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Strong Correlation of Elastin Deletions, Detected by FISH, with Williams Syndrome: Evaluation of 235 Patients

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Summary

Williams syndrome (WS) is generally characterized by mental deficiency, gregarious personality, dysmorphic facies, supravalvular aortic stenosis, and idiopathic infantile hypercalcemia. Patients with WS show allelic loss of elastin (ELN), exhibiting a submicroscopic deletion, at 7q11.23, detectable by FISH. Hemizygosity is likely the cause of vascular abnormalities in WS patients. A series of 235 patients was studied, and molecular cytogenetic deletions were seen in 96% of patients with *classic* WS. Patients included 195 solicited through the Williams Syndrome Association (WSA), plus 40 clinical cytogenetics cases referred by primary-care physicians. Photographs and medical records of most WSA subjects were reviewed, and patients were identified as "classic" (n = 114) or "uncertain" (n = 39). An additional 42 WSA patients were evaluated without clinical information. FISH was performed with biotinylated ELN cosmids on metaphase cells from immortalized lymphoblastoid lines from WSA patients and after high-resolution banding analysis on clinical referral patients. An alpha-satellite probe for chromosome 7 was included in hybridizations, as an internal control. Ninety-six percent of the patients with *classic* WS showed a deletion in one ELN allele; four of these did not show a deletion. Of the *uncertain* WS patients, only 3 of 39 showed a deletion. Of the 42 who were not classified phenotypically, because of lack of clinical information, 25 patients (60%) showed a deletion. Thirty-eight percent (15/40) of clinical cytogenetics cases showed an ELN deletion and no cytogenetic deletion by banded analysis. These results support the usefulness of FISH for the detection of elastin deletions as an initial diagnostic assay for WS.

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Introduction

Patients with Williams syndrome (WS) (Williams et al. 1961; Beuren et al. 1964; Jones 1988) usually present with anomalies including mental deficiency, unusual facies, gregarious personality, congenital heart disease, and often idiopathic infantile hypercalcemia (Morris et al. 1988; Boraz 1991). An example of a characteristic clinical phenotype is shown in figure 1. WS usually occurs sporadically, though autosomal dominant inheritance has been reported (Morris et al. 1993*b*). Variable expression of the phenotype may complicate diagnosis in some patients. Supravalvular aortic stenosis (SVAS), which can also be an isolated autosomal dominant trait, is frequently associated with WS. SVAS as an inherited vascular disease is caused by mutations in the elastin gene (ELN) (Curran et al. 1993; Morris et al. 1993*a*; Ewart et al. 1994). Previous studies have identified linkage between the elastin gene and SVAS in two families, with the location of the gene found, by FISH, to be on chromosome 7 at q11.23 (Fazio et al. 1991; Ewart et al. 1993*a*, 1993*b*; Morris et al. 1993*a*). Investigations of SVAS and WS have suggested that WS is a contiguous-gene disorder in which connective-tissue and vascular abnormalities are caused by the deletion of one elastin allele at the 7q11.23 site (Ewart et al. 1993*a*). The deletion has not been identified by high-resolution cytogenetic analysis. Elastin cosmids were used to confirm the deletion in one allele by FISH (Ewart et al. 1993*a*). Deletions were identified in all of nine affected individuals studied, including familial cases, suggesting that ELN deletion plays a role in the pathogenesis of the disease.

Molecular cytogenetics in patients with sporadic WS was performed to assess the utility of FISH as a clinical diagnostic assay. Comparative genetic evaluation of these patients was categorized according to method of referral and available clinical data. We analyzed a series of 235 patients with known or suspected WS: 195 patients were solicited through the Williams Syndrome Association (WSA); an additional 40 patients were referred to the clinical cytogenetics laboratory at the University of Utah Health Sciences Center for testing. A high detection rate of ELN deletions by FISH was observed, with 96% seen in *classic* WS and 38% seen in referrals by primary-care physicians. We believe that the results of

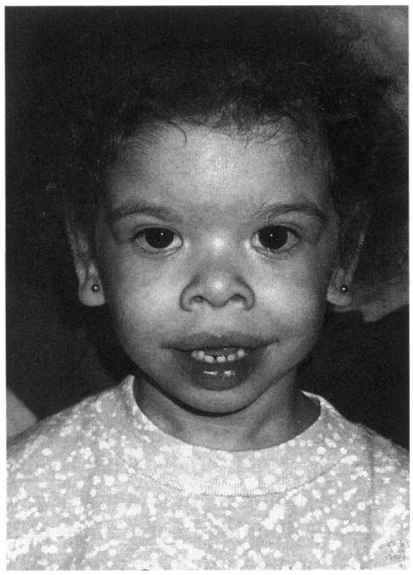

Figure 1 Characteristic facial features of WS: broad brow, periorbital fullness, bitemporal narrowness, low nasal root, flat malar, short upturned nose, full cheeks, full lips, long philtrum, and wide mouth.

this study show that the application of FISH using ELN probes is a powerful diagnostic test in the genetic evaluation for WS.

Subjects, Material, and Methods

A. Patients/Human Subjects

Lymphoblastoid cell lines.—Patients were solicited through the WSA, a national support group with >2,000 members. Peripheral blood was obtained, and Epstein-Barr virus (EBV)–transformed cell lines were established, according to procedures described elsewhere (Neitzel 1986).

Phenotypic analysis of WS patients.—Patients' records and photographs were evaluated by one of us (C.A.M.) and were classified according to our preestablished diagnostic criteria for WS. Patients were classified as "classic" or "uncertain," on the basis of their phenotypic continuum, which included facial features, mental retardation/developmental delay, SVAS, other congenital heart disease, inguinal hernia, and hypercalcemia. Because most patients were not examined in person, a weighted scale was devised in order to classify patients as either "classic" or "uncertain," as shown in table 1. Facial features were scored on the basis of a photograph provided by the patient's family. The other clinical signs were scored on the basis of a review of medical and educational records. The features were chosen on the basis of the likelihood that, if present, they would be included in medical records, and they were weighted according to usefulness as a diagnostic feature of WS. In order to have a positive score for congenital heart disease, either SVAS or other congenital heart disease, documentation by either cardiac catheterization or echocardiography was required. The total possible score was 10. Patients scoring 0–3 were classified as "uncertain," and patients scoring 4–10 were classified as "classic."

Clinical cytogenetics laboratory.—Forty patients were referred to the clinical cytogenetics laboratory at the University of Utah. Clinical information provided with these patients was generally "Rule Out WS." Peripheral blood was processed according to our laboratory's standard protocol for high-resolution analysis and FISH. Cytogenetic reports were issued according to standard laboratory policy.

B. Molecular Cytogenetics

1. Probes.—Cosmid libraries were constructed and screened as described elsewhere (Ewart et al. 1993*a*, 1993*b*). In situ hybridization cosmid probes were labeled with biotin by using a nick-translation kit (Gibco BRL, Life Technologies). The cosmids utilized were cELN-272 and cELN-11D, which are, respectively, 5′ and 3′ to the ELN locus at 7q11.23. We found that the use of both 5′ and 3′ ELN probes provides a much more intense signal than either probe when used alone. Furthermore, previous studies have shown the entire ELN gene to be deleted in WS patients studied (Ewart et al. 1993*b*).

2. FISH.—A repository of EBV-transformed lymphoblastoid cell lines from WS patients were available for use. Metaphase chromosomes from these lymphoblastoid cell lines were prepared according to standard cytogenetic protocol. Slides were made and allowed to air-dry. Slides were baked at 55°C for 1 h prior to use. If the time between harvest and FISH was anticipated to be >1 wk, prepared slides were maintained at −20°C. Hybridization and detection procedures followed the general system previously outlined by other investigators (Pinkel et al. 1988) and the probe manufacturer (Oncor Light Chromosome In Situ System; Oncor), with minor modifications. Biotinylated cosmid probes cELN-11D and cELN-272 were cohybridized with a digoxigenin-labeled control for the pericentromeric region of chromosome 7 (D7Z1; Oncor).

Table 1

Phenotype Scoring System

Phenotypic Feature	Score
Typical facial features	3
Mental retardation/developmental delay	1
SVAS	2
Non-SVAS congenital heart disease	1
Inguinal hernia	1
Hypercalcemia	2
Total	10

Slides were warmed to 37°C immediately prior to use. Warmed slides were treated with RNAse (100 μg/ml; Sigma) at 37°C for 1 h. Slides were washed four times in 2 × SSC (pH 7.0) at room temperature for 2 min each time. Subsequent serial dehydrations followed, for 2 min each in 70%, 80%, and 95% ethanol at −20°C. Slides were air-dried and warmed for 2 min at 56°C. Slide denaturation was carried out at 70°C for 2 min by using 70% formamide/2 × SSC solution. The slides were then immediately transferred to −20°C ethanol in sequence 70%, 80%, 90%, and 100%, for 2 min each. Slides were allowed to air-dry completely, prior to hybridization.

Cosmids cELN-11D (60 ng/μl) and cELN-272 (60 ng/μl), human placental DNA (60 ng/μl), and alpha-satellite probe for chromosome 7 (Oncor) (10 μg/μl) were denatured together and allowed to preanneal for 1 h at 37°C in a hybridization mix of 50% formamide/10% dextran sulfate/2 × SSC. The probe mixture was applied to the denatured/dehydrated, dry slides, coverslipped and sealed. After overnight hybridization at 37°C, the coverslip was removed, and the slides were washed in 50% formamide/2 × SSC at 44°C for 15 min and 2 × SSC at 37°C for 8 min. Slides were rinsed in PN buffer (0.1 M Na_2HPO_4/0.1 M NaH_2PO_4/0.05% Nonidet P-40) prior to detection. For detection of hybridization, slides were incubated in anti-avidin-Cy3 (1 μg/μl in PNM buffer [5% nonfat dry milk {Safeway} in PN buffer]) (indocarbocyanine; Jackson ImmunoResearch Labs) and anti-digoxigenin-FITC (1 μg/μl in PNM buffer) (Boehringer Mannheim) for 30 min. Slides were then washed in PN buffer and counterstained with DAPI/Antifade (Oncor). Slides were maintained in the dark until analyzed. Metaphases were photographed by using either a Zeiss Axioscope or Olympus BH2 epifluorescence microscope, with a double- and triple-band-pass filter (respective excitation/emission: 853 nm/718 nm and 1,570 nm/1,768 nm) (Chroma), and Kodak Ektachrome color-slide film.

Results

Ninety-six percent of patients *who met our criteria* of having *classic* WS were shown to have molecular cytogenetic deletions in ELN. Patients who were not evaluated by our strict phenotyping criteria also showed, by FISH, a high rate of ELN deletions, with 60% of WSA referrals and 38% of clinical cytogenetics cases showing deletions. Representative photographs of hybridizations are shown in figure 2.

Part A: WSA Patients

One hundred ninety-five patients had FISH performed. One hundred fourteen patients were thought to have "classic" WS, 39 patients were classified as "uncertain" WS, and medical records, clinical photographs, or both were not available on an additional 42 patients at the time of this FISH analysis. The results of FISH analysis according to the clinical phenotype are shown in table 2. One hundred ten patients (96%) with the classic phenotype had a deletion of elastin. The specific phenotypic features of these patients are detailed in table 3. The incidence of these clinical features is similar to that in other reported series of patients with WS. The incidence of SVAS is less than that reported in other series, however; not all patients had detailed cardiology evaluation including echocardiogram. The incidence of hypercalcemia was also low, but calcium determinations were available in fewer than half of the patients. The mean score for the 110 patients was 6. By parent report, in no family was there known history of SVAS.

Four patients of the 114 did not have a deletion demonstrable by FISH. Clinical details on these four patients are shown in table 4. Thirty-nine of the patients were classified as "uncertain." Only three in this category were found to have a deletion. All three had mental retardation, and none had either SVAS or hypercalcemia. In the remaining 42 patients, medical information, facial photograph, or both were lacking, and scoring could not be completed.

Part B: Clinical Cytogenetics Case Referrals

Patients referred to the laboratory received a full cytogenetic workup in accordance with policy set by the University of Utah Cytogenetics Laboratory. A total of

Table 2

FISH Results with ELN Cosmids: Correlation with Phenotype

	No. (%) of Cases			
	WSA			
	"Classic"[a] (n = 114)	"Uncertain"[a] (n = 39)	No Clinical Information (n = 42)	Clinical Cytogenetics (n = 40)
Deletion	110 (96)	3 (8)	25 (60)	15 (38)
No deletion	4 (4)	36 (92)	17 (40)	25 (62)

[a] By our graded criteria (refer to Patients, Material, and Methods section).

Table 3

Phenotypic Features of "Classic" WS Patients with Deletion

Phenotypic Feature	No. (%) of Patients
Facial features	110 (100)
Mental retardation/developmental delay	97 (88)
SVAS[a]	53 (48)
Non-SVAS congenital heart disease	56 (51)
Any heart disease[b]	85 (77)
Inguinal hernia	36 (33)
Hypercalcemia	20 (18)

[a] Documented by either cardiac catheterization or echocardiogram.
[b] Includes SVAS and non-SVAS congenital heart disease.

20 metaphase cells were analyzed by trypsin/Wright stain G-banding; 20 metaphase cells were also analyzed for the FISH analysis. Analyses were performed by two independent technologists, for both conventional and FISH studies. Composites were constructed for chromosome 7, as part of the high-resolution analysis (*minimum* banding resolution of 550). Cytogenetic microdeletion at 7q11.23 was not seen in any of the patients analyzed. Of 40 patients examined by FISH, 15 (38%) showed a deletion (ELN signal only on one copy of chromosome 7) (table 2 and fig. 2).

Discussion

In our series of >200 patients, we have shown, by FISH, a strong (96%) correlation of ELN deletions with the clinical phenotype of WS *as assessed by our criteria.* This diagnostic test is now likely to assist in the often difficult clinical evaluation of patients with this syndrome. Diagnosis may be delayed, particularly in those children with mild or absent congenital heart disease, and the arteriopathy present in WS may vary in its clinical expression, over time, in any individual. Facial features of WS may not be recognized in young children or in adults. Appropriate calcium studies are often not performed in young children. The differential diagnosis for WS includes Noonan syndrome and fetal alcohol syndrome, both of which may be associated with developmental delay and congenital heart disease. The discovery of the role of elastin in WS led to the development of a potential laboratory test for the condition.

In the "uncertain" group, three patients were found to have elastin deletions. It is most likely that these patients actually have WS but were misclassified as uncertain on the basis of the available photographs and clinical records. Indeed, one of these patients has since been examined by a clinical geneticist (C.A.M.) and does fit the criteria for WS.

There were four patients in the "classic" category who did not have an elastin deletion detected by FISH. The possible explanations for these findings are as follows: (1) the patients may have had either smaller deletions or mutations of ELN not detectable by the cosmid probes; (2) genes contiguous to elastin may have been deleted that may or may not affect the elastin gene; (3) the four individuals may have had a phenocopy of WS; or (4) the patients may have been misclassified. Identification of genes contiguous to elastin may allow resolution of this question.

Although our data show that no detectable cytogenetic abnormality has been found in WS patients, we still believe that classical cytogenetic analysis with FISH for ELN deletions is the most thorough approach to evaluation of these patients. However, we also believe that *metaphase* FISH alone is likely to provide a high-yield diagnostic test for WS. If no deletion is seen by FISH, it is *imperative* that routine cytogenetic studies be performed. Likewise, if a deletion is detected in a child, we suggest parental studies (at least metaphase FISH) to ensure that no rearrangement involving ELN is present.

We believe that identifying, by FISH, allelic loss of elastin, as an initial laboratory evaluation for WS, provides an accurate means of diagnosing the syndrome. Our finding of 38% deletions by FISH in the clinical cytogenetics cases is intriguing and represents one of the highest-yield cytogenetic tests available. Since many of

Table 4

Phenotypic Features of "Classic" WS Patients without Deletion

Age (years)	Sex	Patient	Facies	Mental Retardation/ Developmental Delay	SVAS	Non-SVAS Congenital Heart Disease	Inguinal Hernia	Hypercalcemia	Total
1.2	F	14573	3	1	2	0	0	2	8
3	F	14960	3	1	2	1[a]	1	0	8
2.2	M	18896	3	1	2	0	0	2	8
1	M	19180	3	0	0	1[b]	0	0	4

NOTE.—For description of scoring, see text.
[a] Supravalvular pulmonic stenosis.
[b] Bicuspid aortic valve.

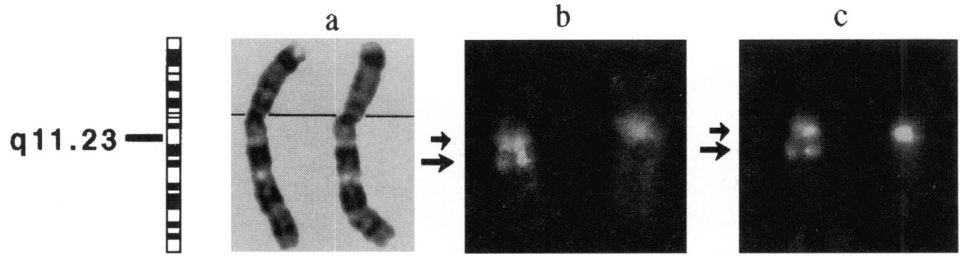

Figure 2 Representation of FISH for WS: ISCN ideogram of chromosome 7, showing ELN location at 7q11.23. *a,* GTG-banded pair of chromosome 7 homologues. *b* and *c,* Metaphase chromosomes from two different patients, showing FISH with a normal chromosome 7 (*left*) and with a chromosome 7 showing ELN deletion (*right*). These gray scale images show the alpha satellite probe for chromosome 7 detected with FITC (*small, upper arrows*) and the ELN cosmids detected with Cy3 (*large, lower arrows*).

these patients were referred without the stringent evaluation of a medical geneticist experienced in the diagnosis of WS, it is anticipated that FISH will provide help in the diagnosis of patients who can then be referred for genetic workup and, ultimately, improved health care. In fact, we propose that clinicians who are not experienced in the diagnosis of WS may wish to consider using our phenotypic classification system as a basis for ordering cytogenetic (i.e., FISH) studies. The small disparity between FISH results and clinical classification, however, stresses the need for continual clinical evaluation and careful correlation with phenotype.

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